Final Report

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"**Molecular dissection of the roles of the SOD genes in mammalian response to low dose irradiation "** was started on **09/01/03** and ended on **08/31/07.** The primary objective of the project was to carry out mechanistic studies of the roles of the anti-oxidant SOD genes in mammalian cellular response to low dose ionizing radiation**.**

Publications and manuscripts accepted for publication in the funding period:

1. Yan, B. Wang, H., Peng, Y., Hu, Y., Wang, H., Zhang, X., Chen, Q., Bedford, J.S., Dewhirst, M.W., and **Li, C-Y.** Apoptotic DNA fragmentation as a checkpoint for genomic instability (2006). *Proceedings of the National Academy of Sciences vol* 103:1504-1509.
2. Yan, B., Wang, H., Li, F., **Li, C-Y.** Regulation of mammalian horizontal gene transfer by apoptotic DNA fragmentation (2006), *British Journal of Cancer*, 96:1696-1700.
3. Chuang, E.Y., Chen,X., Tsai, M., Yan, H., **Li, C-Y.,** Mitchell, JB.,Nagasawa, H., Wilson,PF., Peng,Y., Bedford,JS., and Little, JB. Abnormal Gene Expression Profiles in Unaffected Parents of Patients with Hereditary Type Retinoblastoma. *Cancer Research* (2006) 66: 3428-3433.
4. Yan, B., Wang, H., Zhuo, D., Li, F., Kon, T., Dewhirst, M.W., and **Li, C-Y**. Apoptotic DNA fragmentation factor maintains chromosome stability in a p53-dependent manner. *Oncogene* (Published online April 21, 2006)
5. Yan, B., Wang, H., Rabbani, Z., Zhao, Y., Li, W., Yuan, Y., Li, F., Dewhirst, MW., and **Li, C-Y.** Tumor necrosis factor alpha is a potent endogenous mutagen that promotes cellular transformation (2006), *Cancer Research*, 66:11565-11570
6. Yang, Z., Kon, T., Liu,S., Batinic-Haberle[1],I., Li, C-Y. MnSOD plays key roles in mammalian cellular adaptive response induced by low dose ionizing radiation (2008), *Free Radicals In medicine and Biology, (in review).*

Research Details:

We have used and genetic approach to study the effects of the SOD genes on the biological effects of low dose radiation. The main approach we have used is an siRNA-based approach to knock down SOD1 and SOD2 genes to evaluate their influence on the biological consequences of exposure on

1) We have identified siRNA sequences that can knockdown the expression of SOD1 and SOD2 effectively.
2) Efforts to establish stable cell lines with SOD1 or SOD2 knockdown were not been very successful. Only temporary down-regulation of the genes could be achieved. It appeared that knocking down these genes is detrimental to the host cells. This is consistent with what has been published in literature.
3) In order to achieve efficient knockdown of the SOD genes, we have constructed adenovirus vectors that can express the siRNA sequences rapidly and efficiently. These vectors are very useful to knock down the SOD genes (SOD1 and SOD2) in a rapid and efficient manner in all the cell lines we have tested so far.

4) We have utilized the siRNA-encoding adenovirus to knockdown SOD1 in HCT116 colon cancer cells. The cells were then exposed to low doses of irradiation (10 cGy). The RNA from these cells were then analyzed by DNA microarrays. More than 200 genes were discovered to show > 2 fold difference in gene expression levels.
5) We have examined the relationship between low dose effects and free radical generation in the RPE cells. The RPE (retinal pigment epithelial) cells were chosen because they were of epithelial origin and more relevant for evaluating the carcinogenesis risk of low dose radiation. Some interesting discoveries were made with this approach:
    a. Low dose (10 cGy) has minimal effect on micronucleus generation in RPE cells.
    b. High dose (3 Gy) has a significant effect on micronucleus generation in RPE cells, increasing the background frequency of MN 5 fold over background.
    c. A small (10 cGy) priming dose 5 hours prior to the 3 Gy irradiation significantly reduced radiation induced MN (by >60 %).
    d. The adaptive response is correlated with a significant reduction in level of free radical generation as measured by a fluorescent probe approach.
    e. Consistent with this is that the addition of a novel Mn-TE-2-Porphyrin, which is a novel SOD mimetic synthesized here at Duke, significantly reduced MN induction by radiation. Therefore SOD mimetic has significant potential to be used as countermeasures to the genotoxic and carcinogenic effects of ionizing radiation.

In addition, we found that the low-dose radio-adaptive response is critically dependent on the function of the SOD2 gene. Blocking the SOD2 function through siRNA significantly attenuates the adaptive response in the RPE cells

Summary:
In summary, we conclude that superoxide dismutase genes, especially SOD2, have a significant effect on low dose radiation-induced adaptive response in terms of genetic instability.

Invention and Patents
No patents or inventions were generated from this study.